

REQUESTED REVIEW

Effects of vitamin D in skeletal muscle: falls, strength, athletic performance and insulin sensitivity

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Summary

Accompanying the high rates of vitamin D deficiency observed in many countries, there is increasing interest in the physiological functions of vitamin D. Vitamin D is recognized to exert extra-skeletal actions in addition to its classic roles in bone and mineral homeostasis. Here, we review the evidence for vitamin D's actions in muscle on the basis of observational studies, clinical trials and basic research. Numerous observational studies link vitamin D deficiency with muscle weakness and sarcopaenia. Randomized trials predominantly support an effect of vitamin D supplementation and the prevention of falls in older or institutionalized patients. Studies have also examined the effect of vitamin D in athletic performance, both *inferentially* by UV radiation and *directly* by vitamin D supplementation. Effects of vitamin D in muscle metabolic function, specifically insulin sensitivity, are also addressed in this review. At a mechanistic level, animal studies have evaluated the roles of vitamin D and associated minerals, calcium and phosphate, in muscle function. *In vitro* studies have identified molecular pathways by which vitamin D regulates muscle cell signalling and gene expression. This review evaluates evidence for the various roles of vitamin D in skeletal muscle and discusses controversies that have made this a dynamic field of research.

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Introduction

Long before the discovery of vitamin D, the sun was revered as a source of physical strength and vitality. In ancient Greece,

heliotherapy (sun exposure) was prescribed as a cure for 'weak and flabby muscles'.¹ Sun exposure was considered a performance enhancer; ancient Olympians were instructed to train in sunlight to increase their muscle size.¹

It was in 1922 that the American physician, Alfred Hess, realized that children with rickets had profound muscle weakness and that direct sunlight exposure improved their 'general vigour and nutrition'.² Adolf Windhaus was awarded the 1928 Nobel prize for his work on sterols, including the discovery of the chemical structure of vitamins D₂, D₃ and 7-dehydro-cholesterol.³ The name 'vitamin D' is a historical misnomer. The active form, calcitriol, is a hormone; it is synthesized in humans, undergoes auto-crine regulation and it interacts with a nuclear receptor.⁴ Following the photochemical conversion of 7-dehydrocholesterol to vitamin D under the influence of UV radiation, this molecule circulates bound to vitamin D-binding protein (DBP) and undergoes two hydroxylation steps, firstly in the liver to form 25(OH) Vitamin D (25OHD) and subsequently in the kidney to form 1,25(OH)₂Vitamin D (1,25(OH)₂D) by the 1- α -hydroxylase enzyme (encoded by CYP27B1). 1,25(OH)₂D is the biologically active form that binds to the vitamin D receptor (VDR) to regulate gene expression, with major effects on calcium and mineral homeostasis.⁴ 1,25(OH)₂D regulates its own catabolism *via* upregulation of the expression of CYP24A1 gene (which encodes 24-hydroxylase). Although VDR is present in cell types involved in mineral homeostasis, it is present in other cell types, suggesting that there may be nontraditional roles of vitamin D.

Vitamin D deficiency is now recognized to be common. In Australia, 31% of participants in a study of 11 247 adults were deficient (25OHD < 50 nm)⁵, and an incidence of 41% was recently reported in the United States.⁶ Other parts of the world, such as Asia, India and the Middle East, have also reported high rates of vitamin D deficiency.⁴ With increasing recognition of this pandemic, a plethora of observational studies have associated deficiency with several conditions including autoimmune diseases, diabetes, cancer and muscle weakness.⁷

A direct link between vitamin D deficiency and muscle weakness was first described in the 1960s. In adults with

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osteomalacia, muscle strength improved following supplementation.⁸ Whether this improvement represented a direct effect on muscle or an indirect result of vitamin D-mediated changes in calcium homeostasis was unclear. In the years since this paper, clinical studies examining the effects of vitamin D on muscle performance, mass and the risk of falls in various populations have emerged.^{9,10} There are also a variety of studies that have examined mechanisms responsible for vitamin D's effects in muscle. However, controversy remains regarding the significance of this research and whether the VDR is expressed in adult muscle.^{11–13} In this review, we will cover the clinical evidence for effects of vitamin D in skeletal muscle function and potential mechanisms underlying a role for vitamin D at this site.

Vitamin D and muscle function in older populations

Low muscle mass (sarcopaenia) is characteristic of the ageing process and is associated with a greater risk of disability, falls and mortality in older people.¹⁴ Vitamin D deficiency is common in the elderly and institutionalized and may contribute to sarcopaenia.¹⁵ Reduced capacity for UV-mediated vitamin D synthesis in older skin may be partly responsible. Additionally, VDR expression in human muscle declines with age.¹¹ This may render the muscles of elderly individuals more vulnerable to low vitamin D levels. Higher serum levels may be required to achieve comparable physiological effects. In this section, we discuss observational studies linking vitamin D deficiency and muscle dysfunction in elderly subjects and interventional studies examining potential effects of vitamin D supplementation in the risk of falls and muscle weakness.

Observational studies

Several authors report an association between baseline 25OHD and subsequent falls risk in elderly people. In a prospective, longitudinal study of 1600 elderly women living in assisted care, Flicker and colleagues found a 20% reduction in falls risk over ≈ 150 days with a doubling of 25OHD levels.¹⁶ Similarly, amongst 1231 people over age 65, those with 25OHD levels < 25 nm were more likely to have recurrent falls in the subsequent year (OR 1.78 for ≥ 2 falls).¹⁷

Prospective studies have also reported an association between baseline 25OHD levels and declining muscle function. In a Dutch study of 1000 subjects over age 65, baseline 25OHD level < 50 nm correlated with a greater 3-year decline in physical performance (sum score of walking test, chair stands and tandem stand i.e. ability to stand with feet in tandem position with eyes closed for 10 s).¹⁸ The 3-year risk of sarcopaenia in older subjects was two times greater with baseline 25OHD levels < 25 nm.¹⁵

However, not all studies confirm these associations¹⁹ and despite adjustments, potential confounding factors exist. Low baseline 25OHD levels may be a marker of pre-existing frailty, as the frail elderly are less likely to spend time outdoors, exposed to UV radiation. Malnutrition is common in the

elderly; 40% of those living in institutions are at risk.²⁰ Dietary deficiencies may contribute simultaneously to low vitamin D and to frailty. Factors not considered in adjustment models may confound data interpretation, making interventional studies important.

Intervention studies

Many studies have examined the effect of exposure to UV radiation or vitamin D supplementation in the prevention of falls and muscle strength in older individuals.

Amongst 602 residents of aged-care facilities living in Sydney, Australia, those who increased sunlight exposure had fewer falls than those randomized to the control group over 12 months (incidence rate ratio 0.52, $P = 0.01$).²¹ However, compliance with sunlight exposure was poor (26%), and no effect was observed on intention-to-treat analysis.

Several studies reported beneficial effects of vitamin D on falls. Amongst 625 older residents of assisted-living facilities, those who received calcium (600 mg daily) and ergocalciferol (initially 10 000 IU weekly, then 1000 IU daily) for 2 years were less likely to ever fall than those receiving calcium alone.²² Similarly, in 122 older women in geriatric care, calcium (1200 mg/day) and cholecalciferol (800 IU/day) caused a 49% reduction in falls during the 12-week treatment *vs* the preceding 6-weeks, *vs* calcium alone.²³ Those who fell most had the greatest benefit. Other studies have also suggested benefits in at-risk individuals such as less active older women²⁴ and those suffering stroke.²⁵

Not all studies, however, are concordant.^{26,27} A randomized study of 5292 subjects ≥ 70 years showed no benefit with cholecalciferol (800 IU daily) in the reduction of falls over 26–62 months.²⁷ However, in both negative studies,^{26,27} patients had recently sustained minimal-trauma fracture or were at risk of fracture, suggesting impaired mobility and falls were not the primary study end-point.

One study suggested that 'mega-doses' increased falls risk. An annual oral dose of 500 000 IU of cholecalciferol increased falls in 2256 community-dwelling older women, particularly in the 3 months following the dose (incidence rate ratio 1.31 compared to placebo).²⁸ The mechanism was unclear as serum levels were not toxic. As the vitamin D system is autoregulated by induction of 24-hydroxylase which inactivates 25OHD and 1,25OHD, we speculate that large single doses of cholecalciferol may result in excess 24-hydroxylase induction, rapidly degrading the administered dose and potentially contributing to subsequent deficiency.

Regarding muscle performance, studies show improved lower limb strength following vitamin D supplementation in institutionalized²⁹ and community-dwelling older individuals.³⁰ Those with baseline 25-OHD levels < 50 nm had the greatest benefits. However, a study of single mega-dose therapy (300 000 IU) in 243 frail, older patients did not improve performance even amongst those with baseline levels < 30 nm.³¹ For a detailed list of studies examining vitamin D supplementation and falls, we refer readers to Tables 4 and 5 in a recent review.⁴

Meta-analyses

In the widely cited meta-analysis by Bischoff-Ferrari, a dose-dependent effect of vitamin D supplementation in the reduction in falls was found when examining eight trials and 2426 elderly individuals.³² Compared with placebo, higher doses of vitamin D (700–1000 IU) reduced falls risk by 19%, but lower doses had no effect. Likewise, people who achieved serum 25OHD concentrations ≥ 60 nM reduced falls by 23%, but those who achieved concentrations ≤ 60 nM had no effect.

The recent Institute of Medicine report criticized this meta-analysis for inconsistencies in study inclusion, perceived errors in statistical analysis and reliance on two studies to produce the positive effect, one of which was reportedly not well-powered.³³ On reanalysis with two statistical alterations, the positive effect was not found.

The most recent and largest meta-analysis of 45 782 people, mainly elderly females, found a significant reduction in falls risk amongst those randomized to vitamin D supplementation (OR 0.86 for ≥ 1 fall).³⁴ There was no difference between higher (>800 IU) vs lower doses. Vitamin D was effective in both community-dwelling and institutionalized people with cholecalciferol or ergocalciferol. Reduction in falls was most prominent in patients who were deficient at baseline and with calcium co-administration. In studies included in this meta-analysis, the calcium doses ranged from 500 to 1200 mg daily and elemental calcium and calcium carbonate were used.

Meta-analyses dealing with vitamin D and muscle strength are limited by substantial study heterogeneity in design, including in the parameters of muscle function.³¹ A recent meta-analysis that assessed 17 RCTs involving 5072 participants reported no effect on grip strength or proximal lower limb strength in adults with 25OHD levels >25 nM.¹⁰ However, on pooling data from two studies on vitamin D-deficient adults (i.e. 25OHD < 25 nM), a large effect on hip muscle strength was found although the studies used different measures.

Summary

Although observational studies link falls, muscle weakness and sarcopaenia with vitamin D deficiency, randomized interventional trials have yielded conflicting results. This may be explained by the heterogeneity in study design, variability in treatment dose, duration and the different analyses of falls and muscle function. Nevertheless, the data generally support a benefit of vitamin D supplementation in reducing falls amongst at-risk populations, specifically those with vitamin D deficiency and a history of falls.³⁴ On this basis, we suggest the use of vitamin D in combination with calcium at a daily dose of at least 500 mg for falls prevention in older people with baseline vitamin D deficiency. The dose of vitamin D supplementation and target 25OHD levels are controversial. Whilst the Institute of Medicine recommends 25OHD target levels of 50 nM and daily vitamin D doses of 800 IU d in older adults (>70 years),³³ the US Endocrine society advocates higher serum target level of 75 nM and daily doses of at least 1500–2000 IU in this age group.³⁵ Those

targets are based on skeletal and biochemical outcomes, and the evidence for muscle outcomes does not particularly favour one target over the other. It appears that single-dose mega-therapy is not beneficial for muscle function, and we recommend daily therapy in favour of intermittent mega-dosing.

Vitamin D and muscle function in the young

Children with rickets, a condition characterized by chronic vitamin D deficiency, display profound muscle weakness and hypotonia. Similarly, those affected with the rare condition type II vitamin D dependent rickets display muscle weakness in association with mutations in the VDR.³⁶ These observations suggest that together with a critical role in bone mineralization and metaphyseal fusion, vitamin D is important in muscle development.

Beyond the developmental stage, adults with osteomalacia display proximal myopathy characterized by proximal weakness, pain, a 'waddling gait' and difficulty rising from a seated or squat position.^{8,37}

Here, we review evidence for a role of vitamin D in muscle function and mass in those not yet affected by age-related muscle loss.

Physical performance

There is extensive literature dating back to the 1930s reporting significant improvements in physical performance following exposure to UV radiation. Although these studies do not directly refer to vitamin D, UV-mediated changes in vitamin D status may have played a role in muscle function amongst study participants. In 1938, a Russian group reported significant improvements in 100-m sprint times amongst students receiving UV radiation (7.4% vs 1.7% improvement in controls).³⁸ In 1944, a German group found that medical students receiving UV radiation over 6 weeks had a 13% improvement in performance on a bicycle ergometer.³⁹ In a study from USA, 11 male students experienced a 19% increase in cardiovascular fitness following a course of UV radiation.⁴⁰

Contemporary studies have reported surprisingly high rates of vitamin D deficiency amongst athletes, including Middle-Eastern sportsmen (58% deficient),⁴¹ professional British athletes (57%)⁴² and Australian gymnasts (33%).⁴³ However, studies examining vitamin D supplementation in athletes are sparse. In a study of thirty UK athletes randomized to cholecalciferol 20000, 40000 IU or placebo once per week for 12 weeks, despite significant increases in 25OHD levels at 6 and 12 weeks in the active arms, there was no significant improvement in muscle performance.⁴² In a larger study including 61 male athletes and 30 healthy male nonathletes in the UK, cholecalciferol (5000 IU/day) led to significant improvements in 10-m sprint times and vertical jump over the 8-week period, with no improvement in the placebo group.⁴⁴ Baseline 25OHD levels were lower in this study than in the previous one (mean approximately 40 vs 50 nM), and higher 25OHD levels were achieved (mean 103 vs

approximately 85–91 nM), possibly explaining the discrepancy. With the potential involvement of vitamin D in metabolic pathways,⁴⁵ athletes may require a higher intake to ensure adequate availability for muscle metabolism and physical performance. Once again, there are suggestions that high-dose supplemental vitamin D does not improve muscle function compared with daily lower doses.^{42,44}

A study of 14 recreationally active subjects found an association between baseline vitamin D levels and muscle recovery, assessed by leg isometric force following a bout of intense exercise.⁴⁶ Pre-exercise 25OHD levels predicted immediate and persistent muscle weakness (i.e. 48 and 72 h postexercise), but there was no correlation at 24 h. Interestingly, 25OHD levels initially increased (by approximately 5 nM) and then decreased following exercise, and the authors hypothesized this was due to exercise-related shifts in cytokine and protein levels.

In nonathletes, supplementation studies have yielded mixed results. Amongst 69 adolescent females, those randomized to receive 150 000 IU ergocalciferol orally every 3 months for 1 year demonstrated significant improvements in movement efficiency, a composite of jump height and velocity measured by mechanography, compared with baseline.⁴⁷ Additionally, higher baseline 25OHD correlated with greater jumping velocity.

In another study of 179 vitamin D-deficient adolescent females in Lebanon, those randomized to receive cholecalciferol (doses 1400 or 14 000 IU weekly) did not improve grip strength.⁴⁸ Adequate 25OHD levels were achieved in the high-dose group but not in the low-dose group (\approx 95, 40 nM, respectively, placebo 40 nM), implying that increases in lean mass and bone mineral content seen in both groups *vs* placebo at 1 year were not directly due to serum 25OHD levels.

In a case-control study, 55 veiled Arabic women with severe vitamin D deficiency (mean 25OHD 7 nM) were weaker on all tested parameters of muscle function than 22 Danish women with higher levels (47 nM).⁴⁹ Following vitamin D repletion (IM ergocalciferol: 100 000 IU per week for 1 month then monthly for 5 months and 400–600 IU orally daily), the Arabic women had significant improvements in muscle function and pain at 3 and 6 months. This may have been due to improvement in related biochemical defects and does not prove a *direct* role for vitamin D. In any case, mega-dose supplementation was helpful in this group.

Therefore, vitamin D deficiency is common in young populations, and vitamin D supplementation may improve physical performance in athletes depending on the dose.^{42,44} Such an effect is less clear in nonathletic populations.^{47,48}

Myalgia

Studies examining a putative association between vitamin D deficiency and myalgia (i.e. muscle pain) have generally been small, observational and potentially confounded.

Vitamin D deficiency was reported in 93% of individuals with persistent, nonspecific musculoskeletal pain.⁵⁰ Significantly, more frequent occurrence of 25OHD level <20 nM was found in 40 premenopausal women with fibromyalgia compared with 37 age-matched controls.⁵¹ These studies were observational and

potentially confounded by factors such as physical activity, smoking and body mass index (BMI).

A single randomized trial of ergocalciferol supplementation (50 000 IU weekly for 3 months) in 50 subjects with 25OHD < 50 nM failed to demonstrate improvement in pain scores compared with baseline or placebo.⁵² As the study was adequately powered to detect a significant change in pain, results would suggest that high-dose supplementation has no benefit in the management of generalized pain in the absence of osteomalacia. Therefore, despite an association between vitamin D deficiency and myalgia, the single RCT addressing this question shows no effect.

Vitamin D receptor (VDR) polymorphisms

Vitamin D receptor is the nuclear receptor to which 1,25 (OH)₂D binds to regulate gene expression. Several VDR polymorphisms exist that are associated with a range of biological effects, including bone mineral density and muscle function.⁵³ The FokI polymorphism, found in approximately 35% of Caucasians,⁵³ is a T/C transition in exon 2 that results in a shorter protein with enhanced transactivation capacity. Although this greater activity might suggest improved muscle strength, the FokI polymorphism is in fact associated with reduced muscle strength. In 107 patients with chronic obstructive pulmonary disease (COPD), homozygosity was associated with reduced quadriceps strength compared with heterozygosity.⁵⁴ In another study, the Fok I genotype associated with reduced muscle mass and sarcopaenia in 302 Caucasian men aged 58–93 years.⁵⁵

The BsmI polymorphism affects the 3' region, which is important in the regulation of gene expression. It is reported in approximately 40% of Caucasians.⁵³ In studies of older individuals, the bb genotype was associated with greater strength and reduced risk of falls. Amongst 121 healthy women >70 years, the bb genotype was linked to higher quadriceps and wrist strength compared with the BB genotype.⁵⁶ In two population-based studies from Italy and the UK, the bb genotype of the BsmI polymorphism appeared to protect against falls.⁵⁷

However, in younger people, studies link the bb genotype to *reduced* strength. Amongst 175 women (20–39 years), bb genotype associated with lower hamstring strength and lower fat-free mass compared with the BB genotype.⁵⁸ In a Chinese study of 109 female university students, bb genotype was associated with significantly lower peak torque in concentric knee flexors.⁵⁹

Whilst these polymorphisms are interesting, the associations are not consistent and lack mechanistic explanation. Larger studies to assess the associations are needed. Importantly, polymorphisms of other components of the vitamin D system may also be relevant: DBP polymorphisms have been associated with different responses to vitamin D₃ supplementation⁶⁰, and CYP27B1 polymorphisms have been linked to a greater risk of osteoporotic fracture.⁶¹

Mechanisms linking vitamin D and muscle function

A variety of mechanisms by which vitamin D impacts on muscle cells and fibres have been elucidated. This research includes the

description of muscle morphology in vitamin D-deficient subjects, signalling pathways by which vitamin D effects muscle cells *in vitro*, and *in vivo* studies that have sought to delineate effects and mechanisms of aberrant vitamin D signalling on muscle function. These have been summarized in Fig. 1.

Changes in muscle morphology

Several reports dating back to the 1970s described striking morphological changes in the skeletal muscle of subjects with osteomalacia, likely related to severe vitamin D deficiency.⁶² The main change described is atrophy of the type 2 (i.e. fast twitch) muscle fibres with some reports of scattered necrosis and derangement of the intermyofibrillar network.⁶³

These changes appear to be reversible. Eleven patients with a condition described as 'bone loss of ageing' displayed significant increases in the proportion and cross-sectional size of fast-twitch type IIa fibres of the vastus lateralis following treatment with the vitamin D analogue, 1α -OHD₃ and calcium for 3–6 months.⁶⁴ Interestingly, vitamin D supplementation resulted in changes to the oxidative capacity of muscle – succinate dehydrogenase activity and total phosphorylase activity were low at baseline and increased with treatment whilst lactate dehydrogenase activity, a measure of anaerobic metabolism, did not change.⁶⁴

At a functional level, Sato and colleagues suggested that the reduction in falls in a group of 48 women with poststroke hemiplegia who received vitamin D₂ (1000 IU daily) supplementation could be explained by the increase in the proportion and diameter of type II muscle fibres at 1 year.²⁵ A recent study also implicates improved mitochondrial function as a possible mechanism in the prevention of falls by vitamin D.⁴⁵ In twelve individuals with severe vitamin D deficiency (<15 nm), 10–12 weeks of treatment with cholecalciferol 20 000 IU on alternate days resulted in improved muscle mitochondrial function, as assessed by NMR spectroscopy.

There is an association between fatty infiltration of skeletal muscle and vitamin D deficiency. In 90 postpubertal females in California, the proportion of quadriceps muscle fat, assessed by computed tomography (CT), was strongly inversely correlated with serum 25OHD levels, independent of body mass or subcutaneous and visceral fat.⁶⁵ Similar findings were reported in 20

older subjects who received MRI (magnetic resonance imaging) of the thigh.⁶⁶ Interestingly, selective and near total fatty degeneration of at least one muscle was observed amongst 55% of patients with 25OHD <50 nm.⁶⁶

These studies raise the interesting possibility that vitamin D deficiency may increase the deposition of intramyocellular lipids, either directly or potentially as a consequence of the reduced physical activity or *via* reduced muscle mitochondrial capacity. As defects in mitochondrial fuel metabolism and increases in intramuscular lipids are thought to play a role in the pathogenesis of skeletal muscle insulin resistance,⁶⁷ these findings may potentially explain the association between vitamin D deficiency and insulin resistance.

VDR and muscle

The presence of the VDR in avian, murine and human muscle cells is described by immunohistochemistry, Western blot and detection of VDR mRNA by RT-PCR.^{68–70} However, a recent paper suggested that the VDR is not expressed in skeletal, cardiac or smooth muscle.¹² Differences in experimental conditions and the possibility of tight protein binding of VDR to DNA may have accounted for this finding. Another possible explanation is that VDR is expressed at low levels in resting muscle but is activated by particular conditions. In support of this, a recent paper reported that VDR expression increases following muscle injury in mice.¹³ Interestingly, this paper also reported the presence of 1-alpha hydroxylase (CYP27B1) in muscle and that it *too* is activated during muscle injury. These findings support the intriguing possibility that VDR and CYP27B1 play roles in muscle regeneration and that muscle possesses the capacity for local production of 1,25(OH)₂D.

More recently, a pilot study linked vitamin D supplementation (4000 IU/day for 4 months) with an increase in muscle VDR expression and fibre size in older, mobility-limited women.⁷¹ Although this was a small study and the specificity of the particular antibody used is unclear (VDR-NR1H1), this study provides tantalizing evidence that vitamin D may influence muscle fibre size in humans by upregulating local VDR expression.

Vitamin D receptor defects also influence muscle function. Mice with congenital absence of VDR display a number of deficits in muscle function. On forced swim tests, they display more sinking episodes and fatigue compared with wild-type mice.⁷² These changes are probably largely related to impaired muscle development with generalized atrophy of muscle fibres in this mouse model.⁷³ One important caveat is that these mouse models of VDR defects also display secondary biochemical abnormalities in calcium, phosphate and parathyroid hormone that could affect muscle function.

One study examined the precise aetiology of vitamin D deficiency myopathy by rendering rats vitamin D, phosphorus and calcium deficient by dietary methods.⁷⁴ Phosphorus levels correlated independently with the reduction in soleus muscle force in these rats, and phosphorus repletion in the presence of persistent vitamin D deficiency resulted in complete restoration in slow-twitch muscle force.⁷⁴ Another study reported that calcium

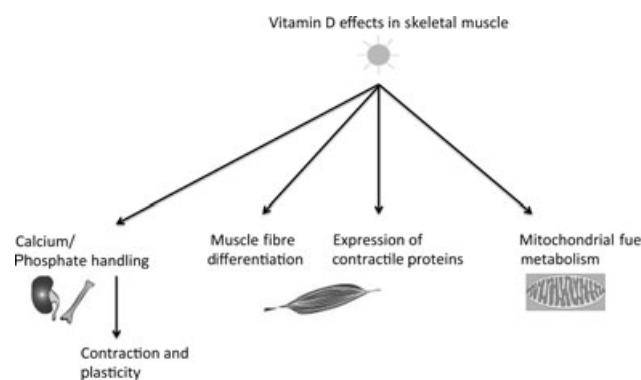


Fig. 1 Potential effects of vitamin D on muscle cells.

was predominantly important in the type 2 muscle fibre atrophy and the muscle protein degradation seen in vitamin D-deficient rats.⁷⁵ Although calcium repletion mostly corrected these abnormalities, persistent vitamin D deficiency was itself associated with a degree of muscle fibre atrophy.

It therefore appears that muscle defects are due, in part, to vitamin D deficiency but predominantly to the associated hypocalcaemia and hypophosphataemia. Repletion with vitamin D remains the most effective treatment as it corrects all of those defects.^{49,76}

Vitamin D and intracellular pathways in muscle

A variety of molecular pathways by which vitamin D may affect muscle cells have been elucidated. These effects may be rapid, occurring within seconds to minutes of vitamin D treatment and include the release of calcium from intracellular stores and its subsequent entry *via* voltage-gated membrane channels.⁴ This suggests a role for vitamin D in the calcium-mediated functions of muscle, namely contraction, plasticity, mitochondrial function, insulin signalling and fuel handling. Whilst evidence for a link between vitamin D and mitochondrial function is only recent,⁴⁵ potential alterations in muscle substrate metabolism may help explain the occurrence of insulin resistance,⁷⁷ intramuscular fatty deposition⁶⁵ and muscle weakness⁴⁹ in vitamin D-deficient subjects. Serum 25OHD levels correlate with recovery rates of phospho-creatinine muscle stores after exercise, suggesting a broader link with ATP production and oxidative function.⁴⁵

Vitamin D could lead to delayed effects on muscle *via* gene expression. This involves binding of the 1,25OHD-VDR-RXR (retinoid X-receptor) complex to vitamin D response elements of DNA and includes effects in the expression of contractile proteins and myogenic transcription factors which influence muscle development.⁷³

More recently, it has emerged that skeletal muscle serves a storage site for 25OHD.⁷⁸ Skeletal muscle was found to express megalin and cubulin, proteins necessary for the endocytic internalization of DBP-bound 25OHD and muscle fibres were noted to retain substantially higher proportions of tritium-labelled 25OHD than bone cells.

Therefore, evolving research points to both rapid and genomic effects of vitamin D in skeletal muscle and its storage at this site that may have a range of effects that remain unknown at this time.

Vitamin D and insulin sensitivity

Apart from the generation of force, skeletal muscle is a highly metabolic tissue that responds to a range of hormones including growth hormone, IGF-1, corticosteroids and insulin. Under normal physiological conditions, skeletal muscle is responsible for approximately 85% of whole-body insulin-mediated glucose uptake⁷⁹ and is therefore of primary importance to insulin resistance. In this section, we discuss the evidence linking vitamin D deficiency with insulin resistance and the broader implications for the pathogenesis of type 2 diabetes.

Observational studies

Amongst 808 nondiabetic participants of the Framingham Offspring Study, plasma 25OHD was inversely associated with fasting insulin concentrations and homeostasis model assessment of insulin resistance (i.e. HOMA-IR) after adjustment for age, sex and BMI (summarized in Table 1).⁸⁰ A similar association between 25OHD and HOMA-IR was found in 712 subjects at risk of diabetes.⁸¹ HOMA is a mathematical model, based on data from physiological studies, which is used to estimate insulin resistance (HOMA-IR) and insulin sensitivity (HOMA-S). In a group of 126 healthy young adults, there was a significant association between 25OHD and insulin sensitivity assessed by hyperglycaemic clamps, after adjustment for a range of factors including BMI.⁸²

Prospective studies show an association between baseline 25OHD levels and the long-term risk of developing insulin resistance (Table 1). In a study of 5200 participants, a 25 nm increment in baseline serum 25OHD levels was associated with a 24% reduced 5-year risk of diabetes.⁷⁷ A positive and independent association with HOMA-S at 5 years was also reported ($r = 0.16$, $P < 0.001$).

These observational studies suggest, but do not prove, a causal or directional relationship between vitamin D status and insulin resistance. Multiple possible confounders exist. In particular, adiposity has an independent inverse association with 25OHD, in many (though not all) studies.⁸³ This may relate to storage of fat-soluble compounds, the avoidance of sunlight exposure and outdoor activity amongst potentially self-conscious obese individuals. Adiposity is a key factor in insulin resistance. Other potential confounders include physical activity, parathyroid hormone levels, serum calcium and dietary intake, all of which may influence or reflect 25OHD levels and also independently influence insulin sensitivity.

Interventional studies

In people without diabetes or underlying insulin resistance, there is no clear evidence that vitamin D supplementation results in improved insulin sensitivity (summarized in Table 2). However, limited evidence suggests that subjects at risk of diabetes may benefit. At-risk subjects randomized to receive various regimens of cholecalciferol and calcium over 6 weeks–3 years, displayed significant improvements in insulin sensitivity, secretion and/or the disposition index (an integrated measure of insulin secretion and action).^{84–86}

The evidence is mixed for subjects with established diabetes. In a recent trial of 90 diabetic subjects, those randomized to receive daily vitamin D (1000 IU) in fortified yoghurt demonstrated improved glycaemic control and insulin resistance (HOMA-IR) *vs* those receiving plain yoghurt.⁸⁷ Importantly, an inverse correlation was observed between changes in serum 25OHD and HOMA-IR in this study.

As summarized in Table 2, the large number of small interventional studies in different populations with baseline vitamin D status, using different modes of supplementation, creates

Table 1. Cross-sectional studies assessing the correlation between serum vitamin D levels and parameters of insulin sensitivity and glucose homeostasis

Study (ref)	Positive or negative	No.	Age, sex	Other	Vitamin D nm (ng/ml)	Tests	Findings	Risk or correlation	Adjustments
Kositsawa ⁹¹	Positive, subgroup	9773	>18, M, F	NHANES		HbA1c	Inverse association 35–74 years	PE = -0.0035	Age, race, sex, DM, activity, supplements, PTH
Scragg ⁹²	Negative	6228	>20 M, F	All	M 78 (31), F 73 (29)	HbA1c	Inverse association non-DM	PE = -0.0014	Age, sex, BMI, activity, season
	Positive, subgroup	2766	>20 M, F	Caucasian	80 (32)	Log (HOMA-IR)	Positive association	β = -0.009	Age, sex, BMI, activity, season
	Negative, subgroup	1736	>20 M, F	African Americans	49 (19)	Log (HOMA-IR)	No association	β = 0.001	Age, sex, BMI, activity, season
	Positive, subgroup	1726	>20 M, F	Mexican Americans	66 (26)	Log (HOMA-IR)	Positive association	β = -0.016	Age, sex, BMI, activity, season
Cheng ⁹³	Negative	3890	40, M, F		92 (37)	Insulins, HOMA-IR	No association		Adipose tissue, season, WC, activity, D intake
Ford ⁹⁴	Positive, subgroup	1941	12–17 M, F		>75 (30) vs <50 (20)	Fasting insulin, HbA1c	Association with insulin in males	24% lower insulin	Age, sex, BMI, HDL, ethnicity, activity, time, supplements, cholesterol
Kayanil ⁸¹	Positive	960	50, M, F	Caucasian	56	HOMA-IR, ISI on OGTT	Both markers associated	r = -0.29	SES, activity, supplements, PTH, BMI
Liu ⁸⁰	Positive, subgroup	808	60 M, F		>54 (22) vs <39 (16)	HOMA-IR, ISI, Insulins at OGTT	Improved fasting insulin and HOMA-IR	(10% vs 13%)	Age, sex, BMI, waist circumference, smoking
Pinelli ⁹⁵	Positive, subgroup	542	38 M, F	46% Ins Res, 42% IGT	M 45 (18), F 35 (14)	HOMA-IR, FPG, HbA1c	Inverse association, men only	M r = -0.19	
Del Gobbo ⁹⁶	Negative	510	>18 M, F	Canadian Cree	52 (21)	HOMA-IR	No association	r = 0.03	BMI, age, sex, activity, education, ETOH, smoking
Gannage' - Yared ⁹⁷	Positive, subgroup	381	24 M, F	Lebanon	77 (31)	HOMA-IR, FPG	Association with FPG	r = -0.15	Sex and BMI
Baynes ⁹⁸	Positive	142	70–88 M		42 (17)	Insulin at OGTT	Inverse association	r = -0.18 to -0.23	Age, BMI, activity, month, smoking, ETOH
Manco ⁸³	Negative	116	F	Italian	39 (16)	EH clamp	No association		
Chiu ⁸²	Positive	126	Approximately 25 M, F		47–70 (19–28)	ISI on H clamp	Significant association	r = 0.25	Age, sex, ethnicity, BMI, WHR, BP, season

BMI, body mass index; BP, blood pressure; EH clamp, euglycaemic-hyperinsulinaemic clamp; ETOH, alcohol consumption; FPG, fasting plasma glucose; H clamp, hyperglycaemic clamp; ISI, insulin sensitivity index; IR, insulin resistance; MetS, metabolic syndrome; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; NR, not reported; PE, parameter estimates; SES, socio-economic status; WC, waist circumference; WHR, waist-to-hip ratio.

Table 2. Interventional studies assessing the effects of vitamin D supplementation on parameters of insulin sensitivity

Study (ref)	Positive or negative	No.	Age, sex	Other	Basal 25OHD nm (ng/ml)	Intervention D in IU/day, Ca in mg	↑ VitD?	Follow-Up	Outcomes	Findings	Adjustments
Nondiabetic at baseline											
De Boer ⁹⁹	Negative	33951	50–79 F		<80 (32) in 89%	D ₃ 400 + Ca 1000 vs placebo	No	7 years	T2D onset	No change	Nil
Nilas et al ¹⁰⁰	Negative	238	45 months 54 F		NR	D ₃ 2000, 1 α -OHD 0.25 μ g, 1,25OHD 0.25–0.5 μ g or placebo	NR	2, 1 years	BGL, weight	No change	Nil
At risk of diabetes											
Pittas ⁸⁶	Positive, subgroup	314	Approximately 71 M, F	92 IFG, 222 NGT	>65 (26) in most	D ₃ 700 + Ca 500 or placebo	Yes	3 years	FPG, HOMA-IR	IFG arm ↓ rises in FPG and HOMA-IR	Age, sex, BMI, activity, smoking
Mitra ⁸⁴	Positive	92	57 M, F	BMI >25, IGT &/or IFG	61 (24.5)	D ₃ 2000 or Ca 800 mg	Yes	16 weeks	Clamp	↑insulin release and DI	Age, sex, BMI, baseline values, race, time
Nagpal ⁸⁵	Positive	71	Approximately 45 M	Central obesity, Indian	<50 (20)	D ₃ 120 000 2 weekly or placebo	Yes	6 weeks	OGIS	Improvement	Age, WHR, baseline 25OHD, betel-nut use
Ljunghall ¹⁰¹	Negative	65	61–65 M	IGT	'Normal'	1 α OHD ₃ (0.75 μ g) or placebo	Yes	3 months	FPG, IVGTT, HbA _{1c}	No change	Nil
von Hurst ¹⁰²	Positive	81	23–68 F	South Asian	<50 (20)	D ₃ 400 or placebo	Yes	6 months	HOMA-IR, Insulin	Improved. More effect if 25OHD >32 ng/ml	Baseline values
Subjects with diabetes											
Nikooyeh ⁸⁷	Positive	90	51 M, F	Iranian	Approximately 44 (18)	Yoghurt: plain vs 1000 D ₃ vs D ₃ 1000 + Ca 300	Yes	12 weeks	HbA _{1c} , HOMA-IR	↓ HbA _{1c} and HOMA-IR	Nil
Sabherwal ¹⁰³	Positive	52	33–73 M, F	South Asian Retro	15 (6)	D ₃ 400 and Ca 1200 No placebo group	Yes	3 months	HbA _{1c}	ΔHbA _{1c} and Δ25OHD correlated. $r = -0.305$	Nil

(continued)

Table 2. (continued)

Study (ref)	Positive or negative	No.	Age, sex	Other	Basal 25OHD nm (ng/ml)	Intervention D in IU/day, Ca in mg	↑ VitD?	Follow-up	Outcomes	Findings	Adjustments
Jorde ¹⁰⁴	Negative	36	Approximately 55 M, F	Met for min + insulin	Approximately 60 (24)	D ₃ 40 000/w or placebo	Yes	6 months	C-peptide HbA _{1c} fasting insulin	No change	Age, sex, BMI, time, baseline D
Borissova ¹⁰⁵	Positive: insulin secretion; negative: IR	27	F	10 T2DM, 17 control	35 (14)	T2DM: Vitamin D ₃ 1332	Yes	1 months	IVGTT, Peripheral IR	↑ 1st-phase insulin. No change in IR	Nil

Ca, calcium; IGT, impaired glucose tolerance; IFG, impaired fasting glucose; FPG, fasting plasma glucose; HbA_{1c}, glycated haemoglobin; IR, insulin resistance; DI, disposition index; OGIS, oral glucose insulin sensitivity; NR, not reported; OGTT, oral glucose tolerance testing; IVGTT, intravenous glucose tolerance testing; HOMA-IR, homeostasis model assessment of insulin resistance; EH clamp, euglycaemic-hyperinsulinaemic clamp; QUICKI, quantitative insulin sensitivity check index; FIRI, fasting insulin resistance index, doses of vitamin D and calcium are daily unless specified otherwise; Retro, retrospective; BMI, body mass index.

difficulty in drawing definite conclusions. Methods of assessing insulin sensitivity range from measuring fasting insulin to euglycaemic-hyperinsulinaemic clamps. Whilst insulin resistance is a factor in the genesis of type 2 diabetes, there is a complex interaction between glucose and insulin levels, with glycaemic outcomes reflecting the relationship between both insulin resistance and insulin secretory capacity.

Thus, the question of whether vitamin D supplementation is effective in the amelioration of insulin resistance, and with particular respect to a role in muscle, remains unanswered. A number of large clinical trials are currently underway, which may definitively answer this question (e.g. NCT00736632, NCT01354964, NCT01315366).

Potential mechanisms

Mouse models of type 2 diabetes show improvements in insulin sensitivity following treatment with 1 α OHD₃.⁴ *In vitro* studies help to explain this link. 1,25D leads to increased expression of insulin receptors and amelioration of insulin resistance *via* effects on Akt and insulin receptor phosphorylation.⁸⁸ Nongenomic effects of vitamin D may also be important. In addition to intracellular calcium regulation, vitamin D leads to the release of arachidonic acid, a polyunsaturated fatty acid, from the cell membrane and into the cytoplasm of muscle cells.⁸⁹ This further links vitamin D with insulin sensitivity as does the possibility that vitamin D may influence caveolin-I,⁹⁰ a scaffolding protein within the membrane that plays roles in metabolism.

Conclusions

Our understanding of the role of vitamin D in skeletal muscle results from the combined efforts of basic scientists and clinical researchers (summarized in Table 3). Reports of profound muscle weakness in children with rickets more than 80 years ago and more recent descriptions of muscle weakness and myalgia in adults with severe vitamin D deficiency have provided *prima facie* evidence of a role for vitamin D in muscle. Although historical literature reports substantial improvements in physical performance amongst athletes exposed to UV irradiation, a direct association with vitamin D status was not established in these early studies and remains to be substantiated by contemporary research.

Amongst older individuals, studies examining the effect of vitamin D on strength and falls have had mixed results. The general consensus is that correction of vitamin D deficiency is beneficial in preventing falls, in combination with calcium supplementation and with the recommended aim of achieving serum 25OHD levels of >50 nm according to the IOM or >75 nm according to the US Endocrine society.

Animal studies have demonstrated that congenital lack of the VDR and models of vitamin D deficiency are associated with defects in muscle function. From these studies, it has been difficult to dissect the individual contribution of vitamin D signalling to muscle function, distinct from its role in mineral homeostasis. At a molecular level, muscle cells show both rapid

Table 3. Conclusions

Vitamin D deficiency is associated with muscle weakness, falls and sarcopaenia

In general, RCT evidence supports the use of supplemental vitamin D to prevent falls in elderly, institutionalized individuals

RCT evidence suggests a beneficial effect of supplemental vitamin D in proximal muscle strength in subjects with severe vitamin D deficiency.

However, this area is clouded by nonuniformity in end-points and muscle parameters

Although vitamin D deficiency is associated with myalgia, the single RCT addressing this question shows no effect of vitamin D supplementation in alleviating myalgia in deficient subjects

Vitamin D deficiency is surprisingly common in athletes. Two recent RCTs suggest that higher serum 25OHD levels (approximately 100 nM) may be necessary to produce effects in athletic performance

Vitamin D deficiency is associated with insulin resistance, but this is confounded by adiposity and sedentary, indoor living. There is limited RCT evidence examining the effects of supplemental vitamin D in those with/at risk of insulin resistance, but more studies are currently underway

VDR polymorphisms are associated with differences in muscle function. This weighs in to the debate regarding the presence of VDR in skeletal muscle

Animal studies support a combined role for vitamin D and its associated minerals (calcium/phosphate) in the development of muscle defects in vitamin D deficiency. However, mineral defects appear to be important

In culture, muscle cells respond directly to 1,25(OH)₂D supporting a molecular basis for its *in vivo* effects

Two intriguing studies have recently emerged, one reporting the activation of VDR in regenerating muscle and another demonstrating that muscle serves as a storage site for 25OHD. The elucidation of vitamin D's effects in muscle promises to remain a vibrant and evolving field

VDR, vitamin D receptor.

and delayed responses to the active form of vitamin D, 1,25(OH)₂D, that provides mechanistic support for these clinical and animal studies. Recent research showing the activation of VDR in regenerating muscle and the storage of 25OHD in skeletal muscle raise intriguing questions regarding its unknown roles at this site. In conclusion, vitamin D supplementation prevents falls in susceptible populations, vitamin D pathways regulate muscle development in animal models and vitamin D signalling alters various molecular pathways in cultured muscle cells. Further clinical and translational studies are needed and will be of particular interest in muscle training, insulin resistance and muscle regeneration.

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